Bayer HealthCare
Bayer Schering Pharma

Department: GDD-GED Toxicology

☐ Research Report
☐ Product Report
☐ Development-Product Report
☐ Methods Report
☒ Toxicology Report

Report No.: AT06079

Test Item: **PES Vorstufe 2342**

Title: **Acute toxicity in the rat after dermal application**

Study No.: T 5081835

Author(s): U. Gillissen

Study Completion Date: October 14, 2010

Performing Laboratory:

Bayer Schering Pharma AG
GDD-GED Toxicology
42096 Wuppertal
Germany

Sponsor:

Bayer MaterialScience AG

51368 Leverkusen
Germany

GLP Compliance Statement

This study was conducted in compliance with the OECD Principles of Good Laboratory Practice as revised in 1997 (ENV/MC/CHEM(98)17) and with the revised German Principles of Good Laboratory Practice according to Annex I German Chemicals Act (Bundesgesetzblatt, Volume 2008, Part I, No 28, 1173-1184, issued July 11, 2008):

U. Gillissen — Oct. 9, 2010

U. Gillissen
Study Director

Date

Quality Assurance Statement

Study No.: T5081835

Test Item: PES Vorstufe 2342

On the dates given below inspections were conducted by the Quality Assurance to ensure that no deviations exist that are likely to affect the integrity of this study.
The Quality Assurance Unit monitors the conduct of each study by study-based inspections or by process-based inspections of a similar type of study if the short-term nature of a study precludes inspection while it is in progress. Routine procedures and the equipment used in the relevant laboratory areas are inspected regularly and reports are made in accordance with current SOPs.

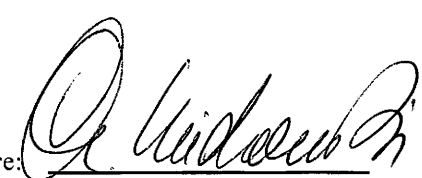
*(study plan amendments, if any, were duly audited and reported to the Study Director and Management)

Date of Audits / Inspections	**Phases Audited / Inspected**		**Date of Report to Study Director and Management**
Aug-13-2010	Study Plan *		Aug-13-2010
Aug-18-2010	process based	Administration / Dosing, Clinical Observation, Raw Data / Documentation, Preparation of Formulation, Weighing	Aug-18-2010
Oct-04-2010	Main Report	1. Draft	Oct-04-2010
Oct-08-2010	Main Report	Final Draft	Oct-08-2010

The results of this study including the methods used have been checked on the basis of the current SOPs.
They have been correctly reported and the report reflects the raw data.
In case of a multi-site study audits at the test sites are presented in the QA Statement of the Principal Investigator's report (see appendix).

Quality Assurance Unit
Global R&D Quality, GLP-Mgmt.

Date: Oct-08-2010

Signature: 
Christina Kiedrowski

Signatures

Study Director: October 14, 2010 (Date) U. Gillissen (U. Gillissen)

Test Facility Management: October 14 2010 (Date) C. Stark (Dr. C. Stark)

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List of Abbreviations

In addition to the abbreviations for the basic units of the International Unit System (SI) stipulated by law, and designations for decimal multiples and parts of units, the following abbreviations are used:

a.m.	(ante meridiem) = before noon
approx.	approximately
bw	body weight
C.A.	Chemical Abstracts
CAS	Chemical Abstracts Service
d	day
E	final necropsy / Endsektion
e.g.	exempli gratia (= for example)
ff	following
h	hour
LD50	median lethal dose
m	mean
M	moribund sacrifice
max. intens.	maximum intensity
n.a.o.	no abnormality observed
no.	number
part.	partly
p.m	(post meridiem) = after noon
ct/CT	dermal / cutan
SD/s	standard deviation
T	death / Tod
time of d.	time of death
ta	treatment area
'	minute
%	percent
♀	female
♂	male

1. Summary

This study was performed to assess the acute dermal toxicity to Wistar rats of PES Vorstufe 2342 (content: 100%).

The results are summarized in the table below.

Table 1-1 Dose-Response

dose mg/kg bw	toxicological result*	occurrence of signs	time of death	mortality (%)
male				
2000	0 / 0 / 5	--	--	0
female				
2000	0 / 0 / 5	--	--	0

* number of animals which died spontaneously and/or were sacrificed in moribund state / number of animals with signs of toxicity / total number of animals used per group

Based on the present investigations, PES Vorstufe 2342 is regarded to have the following LD50 values:

LD50 rat, male : > 2000 mg/kg body weight

rat, female : > 2000 mg/kg body weight

So it is regarded as non-toxic after dermal application.
(GHS Category 5/unclassified analogous OECD draft guideline 434).

Groups of 5 male and 5 female Wistar rats received a single dermal dose of 2000 mg/kg body weight of the test item applied semiocclusively for 24 hours.

A dose of 2000 mg/kg body weight was tolerated by male and female rats without mortalities, clinical signs, effects on weight development and gross pathological findings.

2. Introduction

The study objective was to determine acute toxicity after dermal application. Information derived from this test serves to indicate the possible existence of hazards likely to arise from short-term exposure by the dermal route of the test substance, and - with respect to a proper handling and use - serves to permit classification (labeling) of a product.

3. General Information

The study was sponsored by Bayer MaterialScience AG,
51368 Leverkusen, Germany.
The study was performed at Bayer Schering Pharma AG, GDD-GED General Toxicology, 42096 Wuppertal, Germany.

3.1 Responsibilities

Study Director	U. Gillissen
Test Facility Management	Dr. C. Stark
Head of Test Facility	Dr. F.-W. Jekat
Archiving	R. Zils
Head of Quality Assurance Unit	Dr. A. Paeßens

3.2 Key Study Data

Study No.	T 5081835
Study initiation date	2010-08-12 (YYYY-MM-DD)
Experimental starting date	2010-08-25 (YYYY-MM-DD)
Experimental completion date	2010-09-08 (YYYY-MM-DD)
Study completion date	see signature page

3.3 Archiving

The study protocol, raw data and final report are retained in the archives specified by the test facility Toxicology of the Bayer Schering Pharma AG in Wuppertal. A retention sample of the test item, and, if applicable, also of the reference item is stored in the archive of the test facility.

4. Material and Methods

4.1 Guidelines

The method used complied with the OECD-Guideline for Testing of Chemicals No. 402, "Acute Dermal Toxicity", adopted: 24 February, 1987; EEC Directive 440/2008 Part B, Method B.3., test methods pursuant to Regulation (EC) No 1907/2006 (REACH).

4.2 Test Item

Test item:	PES Vorstufe 2342
Synonym(s):	Ester Rizinus + Sojaoel-Umesterung
EC No.:	919-697-6
Chemical name:	Castor Oil, reaction product with Soybean Oil
Batch no.:	LB06603520
Appearance:	light yellow liquid
Content of test item*:	100 %
Storage*:	refrigerator
Expiry date:	2010-10-22

*due to product information given by the sponsor

4.3 Exposure Procedure

One day before the start of the treatment the back and flanks of the rats were shorn (approximately 10% of the body surface area).
The dosing is based on the test item. The content is not considered.

For each dose and animal the required amount of the pure liquid test substance was calculated on the base of the body weight at time of dosing. This amount was weighed and applied as uniformly and thinly as possible to the test area, covered with a gauze-layer (6.0 cm x 5.0 cm = 30.0 cm^2) of a „Cutiplast® steril" coated with air-tight „Leukoflex®". The gauze strip was placed on the rat's back and secured in place using „Peha®-Haft" cohesive stretch tape and additionally covered with a "Lomir biomedical Inc rat jacket", which was connected with a safety pin to the stretch tape to ensure that the animals could not ingest the test substance.

After approximately 24 hours the dressings were removed and the area was rinsed with tepid water using soap and gently patting the area dry.

4.3.1 Application Dose and Exposure Period

Depending on the body weight of the animals and the surface area on which the test substance was applied, the following dose range (mg/cm^2) was applied (exposure lasted for 24 hours):

Table 4-1 Dose Range

dose (mg/kg b.w.)	surface area	range of doses (mg/cm^2)
male		
2000	30.0 cm^2	19.5 - 19.9
female		
2000	30.0 cm^2	13.3 - 13.7

4.4 Number of Animals and Dose Levels

At start of the study 5 males and 5 females were used for dosing with the start dose, which was selected in dependence with the characteristics of the test item.

4.5 Experimental Animals and Housing Conditions

The study was performed in Wistar rats. The strain used was HsdCpb:Wu (breeder: Harlan GmbH, 5960 AD Horst, Netherlands). Animals of this strain have been used at Bayer Schering Pharma AG for toxicological studies for many years. Historical data on their physiology and spontaneous alterations are available. The state of health of the breeding colony is routinely spot-checked for the main specific pathogens. The results of these examinations are archived.

At start of the study the animals were nulliparous and non-pregnant and free of all clinical symptoms or diseases. The acclimatization time in the animal room was at least 5 days.

Body weights at start of study:

♂ 292 g - 298 g

♀ 200 g - 205 g

This is according to an age of 9 - 13 weeks approximately.

The animals were assigned to their groups by randomization. The random list was based on evenly distributed chance numbers especially generated for the study by a software application. The animals were identified by labels on the cages stating study number, test item, animal number, group number, etc. and by individual animal identification using permanent skin marking.

4.5.1 Husbandry and Nutrition

The animal room had a standardized climate:

Room temperature	22 ±2°C
Air humidity	55 ±5%
Ventilation	approx. 10 changes per hour
Light/Dark cycle	12 hours rhythm

Occasional deviations from these standards occurred, e.g. during cleaning of the animal room. They did not have any apparent influence on the outcome of the study. The animal room was provided with sound from a radio program.

The animals were caged individually in polycarbonate cages on low dust wood granulate bedding (Lignocel BK 8-15, Firma Rettenmaier, Germany). The cages of the animals were placed on racks. The wood granulate was randomly checked for contaminants at regular intervals and the results have been stored at the Department for Laboratory Animal Services, Bayer Schering Pharma AG, 42096 Wuppertal, Germany. The analyses yielded no evidence of any adverse effects on the aim of the study. Wooden blocks for environmental enrichment were added to each cage. As soon as necessary, they were replaced by new ones. The cages were changed at least once a week. Feed racks and water bottles were not changed. All cage material was washed with hot water. In the first stage of the washing programs an alkaline cleaning agent (Neodisher Alka 300; Chemische Fabrik Dr. Weigert GmbH & Co. KG, concentration: 2.2 g/l) was used.

The animals received the standard diet "Provimi Kliba 3883 PM S15 Maus/Ratte Haltung, Kaiseraugst Switzerland", and tap water ad libitum from polycarbonate bottles.

The nutritive composition and the contaminant content of the standard diet were checked and analyzed routinely in random samples. Nothing untoward was found. The tap water was of drinking water quality (according to the Drinking Water Decree in the current version). The results of the analyses have been stored at Bayer Schering Pharma AG, 42096 Wuppertal, Germany. The available data yielded no evidence of any adverse effects on the aim of the study. The food was available from racks in the lid of the cage, polycarbonate bottles were used for drinking water.

The animal room was cleaned and disinfected weekly. A continuous pest control was performed using a cockroach trap without pesticides (e.g. Killgerm Roach Trap, Killgerm GmbH, 41460 Neuss, Germany). The contact of the animals with the traps was avoided in any case.

4.6 Observations

Clinical signs and mortality rates were determined several times on the day of application and subsequently at least once daily for an observation period of at least 14 days. Mortality and in the event of symptoms occurring, nature, duration and intensity (possible grading: no intensity specified / 1 = slight / 2 = distinct) were recorded individually. The day of application is defined as day 1. Times after application until the following day were recorded either in minutes or in hours, depending on what was appropriate. According to international agreements minutes are given in 5-minute intervals (0' - 2.4' is given as 0', 2.5' – 7.4'is given as 5' and so on). Hours were rounded to full hours. In contrast to this, all further observation intervals are given in days. The duration of the symptoms and the times of death are given relative to the time of application to the individual animal. The real time points can be taken from the raw data. In general, death was taken as a symptom. Due to the computer system used, death is not shown as a clinical symptom in the lists of the appendix. If no symptoms were seen until death, time of death was taken as the first occurrence of a symptom. In the results section, the findings are

summarized without any indication of intensity. The findings can be found for the groups and individual animals in the appendix.

The weight gain of the animals was checked weekly until the end of the study. The weights are given in the tables in the appendix as individual and mean values. The weight gain of the animals was calculated based on rounded individual values. The weights are given in grams (g). Indicated under the heading day 8 are e.g. the data obtained on the 7th day after application.

Animals which died or were killed in moribund state were weighed (except on day of application) and dissected as soon as possible, and examined macroscopically. The surviving animals were sacrificed by carbon dioxide at the end of the study, dissected and examined macroscopically.

4.7 Collection, Processing and Evaluation of Data

During this study for collection, storage and evaluation of data a validated LAN-linked computer system was used, which is designed and created in-house. If necessary the data were collected offline.

Hardware and operating systems:

- HP-1000 A (4x, operating system RTE-A 6.2)
- HP-3000 series 900 (1x, operating system MPE/iX 5.5)
- HP-9000 series 200 (1x, operating system HP-UX 11.0)

Software: Data was stored on HP-3000 in an HP TurboImmage/XL Database.

4.7.1 Calculation of the LD50

Only the limit dose of 2000 mg/kg body weight was tested. A judgment according the Global Harmonized System (GHS) was done analogously the OECD draft guideline 434.

5. Results

5.1 Dose-Response Table (LD50)

The results of the study for acute dermal toxicity in the rat, including the LD50, are summarized in the table below.

Table 5-1 Dose-Response

dose mg/kg bw	toxicological result*	occurrence of signs	time of death	mortality (%)
male				
2000	0 / 0 / 5	--	--	0
female				
2000	0 / 0 / 5	--	--	0

* number of animals which died spontaneously and/or were sacrificed in moribund state / number of animals with signs of toxicity / total number of animals used per group

LD50 male : > 2000 mg/kg bw
female : > 2000 mg/kg bw
(GHS Category 5/unclassified analogous OECD draft guideline 434)

5.2 Clinical Signs

No clinical signs were observed (see groups on page 20, individual animals on page 21).

5.3 Body Weights

There were no toxicologically significant effects on body weight or body weight development in males and females.

The weights are given in the appendix as individual and mean values on page 22, for body weight gain see page 23.

5.4 Gross Pathology Findings

The necropsies performed at the end of the study revealed no particular findings (see appendix, page 24).

6. Conclusion

Based on the present investigations, PES Vorstufe 2342 is regarded to have the following LD50 values:

LD50	rat, male	:	> 2000 mg/kg body weight
	rat, female	:	> 2000 mg/kg body weight

So it is regarded as non-toxic after dermal application.
(GHS Category 5/unclassified analogous OECD draft guideline 434).

Ministerium für Arbeit, Gesundheit und Soziales
Des Landes Nordrhein-Westfalen

Fürstenwall 25, 40219 Düsseldorf Aktenzeichen II A 5 – 31.11.46.06

Gute Laborpraxis/Good Laboratory Practice
GLP-Bescheinigung/Statement of GLP Compliance
(gemäß/according to § 19b Abs. 1 Chemikaliengesetz)

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 88/320/EG wurde durchgeführt in:

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 88/320/EEC at:

☒ Prüfeinrichtung/Test facility ☐ Prüfstandort/Test site

Bayer HealthCare AG
BSP-GDD-GED
Toxikologie
Aprather Weg 18 a
42096 Wuppertal

Prüfungen nach Kategorien (gemäß ChemVwV-GLP Nr. 5.3/OECD guidance)	Areas of Expertise (according ChemVwV GLP Nr. 5.3/OECD guidance)
Kategorie 1 Prüfungen zur Bestimmung der physikalisch-chemischen Eigenschaften und Gehaltsbestimmungen	**category 1** physical-chemical testing
Kategorie 2 Prüfungen zur Bestimmung der toxikologischen Eigenschaften	**category 2** toxicity studies
Kategorie 3 Prüfungen zur Bestimmung der erbgutverändernden Eigenschaften (in vitro und in vivo)	**category 3** mutagenicity studies
Kategorie 9 Biochemische Toxikologie; Kurzzeitkanzerogenese; Immuntoxikologie; Sicherheitspharmakologie	**category 9** biochemical toxikology; short time cancerogenicity; immunotoxicity; safety pharmacology
Datum der Inspektion 01.Sept.2008 bis 05.Sept.2008	**Date of Inspection** September 1st 2008 until September 5th 2008

Die/Der genannte Prüfeinrichtung/Prüfstandort befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.

Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung/diesem Prüfstandort die oben genannten Prüfungen unter Einhaltung der GLP-Grundsätze durchgeführt werden können.

Düsseldorf, den 09.02.2009
Im Auftrag

(Dr. Deden)

The above mentioned test facility/ test site is included in the national GLP Compliance Programme and is inspected on a regular basis.

Based on the inspection report it can be confirmed, that this test facility/test site is able to conduct the aforementioned studies in compliance with the Principles of GLP.

Land Nordrhein-Westfalen 13 Ministerium für Arbeit, Gesundheit und Soziales

Dienstsiegel/official-seal

T5081835 clinical signs; groups Akut/acute ID13295/10

clinical signs	incidence	duration of signs	max. intens.	time of death
2000 MG/KG male CT				
n.a.o.				
2000 MG/KG female CT				
n.a.o.				

T5081835 clinical signs; individual Akut/acute ID13275/10

animal no.	clinical signs	duration of signs	max. intens.	time of death
	2000 MG/KG male CT			
1	n.a.o.			
2	n.a.o.			
3	n.a.o.			
4	n.a.o.			
5	n.a.o.			
	2000 MG/KG female CT			
6	n.a.o.			
7	n.a.o.			
8	n.a.o.			
9	n.a.o.			
10	n.a.o.			

T5081835 Tiergewichte / body weights (G) Akut/acute 13245/10

Tiernr./ animalno.	Tag / day 1	8	15	nach Tod after death	Todeszeit time of d.
	2000 MG/KG männlich / male CT				
1	296	320	342		
2	292	313	334		
3	296	321	349		
4	294	328	362		
5	298	328	361		
m	295	322	350		
s	2.3	6.3	12.1		
	2000 MG/KG weiblich / female CT				
6	200	210	220		
7	200	211	212		
8	200	206	210		
9	204	209	219		
10	205	210	212		
m	202	209	215		
s	2.5	1.9	4.6		

T5081835 Gewichtsentwicklung / weight gain (G) Akut/acute 13265/10

Tiernr./ animalno.	Tag / day 1	8	15	Gesamtgew.-Entw./ total weight gain
2000 MG/KG männlich / male CT				
1	296	+24	+22	+46
2	292	+21	+21	+42
3	296	+25	+28	+53
4	294	+34	+34	+68
5	298	+30	+33	+63
m	295	27	28	54
s	2.3	5.2	6.0	11.0
2000 MG/KG weiblich / female CT				
6	200	+10	+10	+20
7	200	+11	+1	+12
8	200	+6	+4	+10
9	204	+5	+10	+15
10	205	+5	+2	+7
m	202	7	5	13
s	2.5	2.9	4.3	5.0

Individual macroscopic findings

All findings

animal no.	time / type of death	finding
group 01	2000 MG/KG male CT	
1	15d / E	General observations
		no pathological finding
2	15d / E	General observations
		no pathological finding
3	15d / E	General observations
		no pathological finding
4	15d / E	General observations
		no pathological finding
5	15d / E	General observations
		no pathological finding
group 02	2000 MG/KG female CT	
6	15d / E	General observations
		no pathological finding
7	15d / E	General observations
		no pathological finding
8	15d / E	General observations
		no pathological finding
9	15d / E	General observations
		no pathological finding
10	15d / E	General observations
		no pathological finding